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CHRONIC INHALATION EXPOSURE OF EXPERIMENTAL ANIMALS TO METHYLCYCLOHEXANE

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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Four animal species were exposed for one year to selected vapor concentrations of methylcyclohexane (MCH) to determine its long-term toxic effects. Year-long exposures of animals were conducted to MCH at 0, 400, and 2000 ppm. Rats, mice, and hamsters were held for one year postexposure while dogs were held for five years postexposure. Mean body weight depression was observed in the MCH exposed hamsters and male rats. The only significant lesions noted in any of the animals was progressive renal nephropathy seen in virtually all of the male rats.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document constitutes a final technical report on MCH. The research covered in this report began in August 1978 and was completed in July 1984 and was performed in part under Air Force Contract Numbers F33615-76-C-5005 and F33615-80-C-0512. K. C. Back, Ph.D. and M. K. Pinkerton served as contract technical monitors for the Air Force Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D. served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor, Department of Community and Environmental Medicine. Acknowledgement is made to M. Majdan, G. L. Fogle, J. C. Welch, C. D. Flemming, J. A. Brewer, and R. K. Blasingame for their significant contributions and assistance in the preparation of this report.

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INTRODUCTION

Methylcyclohexane (MCH) is a liquid hydrocarbon used as a solvent for cellulose ethers and is also found in the aircraft fuel designated JP-9. This fuel is a mixture of three primary ingredients, JP-10, RJ-5, and MCH. JP-10 and RJ-5 are high density hydrocarbons yielding a greater BTU output than conventional aircraft fuels. RJ-5 is a high viscosity fluid which causes pumping and flow problems at low temperature. These problems are corrected by the addition of MCH to the mixture.

Acute toxicity studies of MCH were reported by Treon et al. (1943). Six-hour acute exposures of rabbits to inhaled concentrations of MCH above 10,000 ppm caused convulsions, light narcosis, labored breathing, salivation, and conjunctival congestion. Between 5500 ppm and 7300 ppm, lethargy and impaired coordination were the only signs. Lazarew (1929) reported that 7500 to 10,000 ppm vapor for two hours produced narcosis in mice while 10,000 to 12,000 ppm caused death. Lehman and Flury (1943) indicated that the acute toxicity of MCH was greater than that of heptane, but less than that of octane. Similar high level narcotic effects were reported when mice were exposed to heptane vapors between 10,000 and 15,000 ppm (Fuehner, 1921). In addition, Patty and Yant (1929) reported slight dizziness in man after exposure to 1000 ppm for six minutes. Concentrations of 2000 to 5000 ppm resulted in marked vertigo, hilarity, incoordination, and nausea which persisted for several hours after exposure. The American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH in 1976 from 500 ppm to 400 ppm or 1600 mg/m^3. The recommended short-term exposure limit (STEL) is 500 ppm or 2000 mg/m^3 based on analogy to the toxicity of heptane, and the TLV and STEL values are identical to the TLV and STEL of heptane.

The use of analogy with other solvents for setting human exposure limits has resulted in serious occupational disease outbreaks in exposed workers. A TLV of 500 ppm had been set for n-hexane based solely on acute toxicity data in animals for other petroleum solvents such as pentane. Reports of peripheral neuropathy in workers exposed to hexane resulted in the lowering of the American Conference of Governmental Industrial Hygienists TLV to 100 ppm in 1974 and to 50 ppm in 1980.

Since methylcyclohexane is a highly volatile liquid, exposure to JP-9 would be expected to result in chronic exposure of fuel handlers to fairly high vapor concentrations. Consequently, these studies were undertaken to obtain the data needed to assess

the safety margin of current exposure limits for methylcyclohexane. The design of the study also provided for the detection of oncogenic potential of methylcyclohexane. Animal exposure concentrations of MCH for this study were selected on the basis of the current TLV (400 ppm) and the maximum tolerated level for repeated exposures which appeared to be 2000 ppm.

MATERIALS AND METHODS

Test Agent:

The methylcyclohexane used in this study was manufactured and obtained from Eastman Organic Chemical Corporation. The MCH supplied came in steel drums and consisted of 3 different lots designated A8, A9, and B8. Purity analyses were conducted on the MCH in each drum. The results are tabulated below:

Lot No.	% Purity	% N-Heptane	% Toluene
A8	98.57	0.86	0.56
A9	98.50	0.97	0.52
B8	98.66	0.74	0.60

Only two impurities, n-heptane and toluene, were identified and the relative purity of the MCH was consistent from drum to drum within lots and between lots used in the study.

Generation:

The generation of desired chamber concentrations of MCH was accomplished by metering liquid MCH directly into the chamber inlet air supply stream where vaporization was accomplished in sufficient air volume to prevent formation of an explosive vapor mixture. The liquid was delivered from a drum using 3-5 psig air pressure with dual regulators to prevent overpressurization. Delivery into the air supply line was metered and controlled with a glass flowmeter and 1° needle valve installed on a manifold from the storage drum and housed in an exhaust hood to prevent leakage into work areas. The stainless steel supply lines were wrapped with electrical heating tape to provide modest heat when necessary to prevent recondensation. Generation of MCH was started at a high rate and then adjusted to a steady rate to achieve 95% of the nominal chamber concentration within 15 minutes of daily start-up of animal exposures.

Analysis:

Air samples were continuously drawn from the chambers during animal exposures for analysis using a total hydrocarbon analyzer. Each pair of chambers with the same nominal concentration were sampled alternately on a 15 minute cycle with a single analyzer.

Animals:

Fischer 344 rats (CDF [F344]/CrlBR)[1], Golden Syrian hamsters (Lak:LVG ([SYR])[1], C57BL/6J mice[2] and purebred beagle dogs[3] were used in this study. Rats were ten weeks of age, mice 8 weeks of age, and hamsters 12 weeks of age at the onset of the study. The beagle dogs ranged in age between 8 and 13 months. Food was available only during nonexposure periods while water was available ad libitum.

Clinical Tests and Observations:

The animals used in this study were observed hourly during the one-year exposure phase and at least six times daily during a one-year postexposure period. Rats and hamsters were weighed at biweekly intervals during exposure and monthly during the post-exposure period. Dogs were weighed at biweekly intervals during exposure and biannually during the five-year postexposure period. Mice were weighed in groups with group mean weights followed on a monthly basis throughout the experimental period.

Blood samples were taken from all dogs at biweekly intervals during exposure and every six months postexposure. Blood samples were drawn from rats only at necropsy following the one-year exposure. Clinical determinations were performed for a battery of tests including routine hematology, electrolytes, glucose, creatinine, bilirubin, serum protein, albumin, and three enzymes, SGPT, SGOT and alkaline phosphatase. Organ weight data were also obtained and evaluated for rats and dogs at sacrifice. All of the animals used in this study were necropsied at death with a battery of approximately 33 tissues sampled from each animal for histopathology examination following the protocol used by the National Cancer Institute (Sontag et al., 1976).

[1] Charles River Breeding Laboratory, Wilmington, Massachusetts.
[2] Jackson Laboratories, Bar Harbor, Maine.
[3] Ridglan Farms, Inc., Mt. Horeb, Wisconsin.

Exposure Conditions:

The animals were exposed to MCH by the inhalation route in dome shaped, 840 cubic foot chambers described by Thomas (1965) for one year using an industrial work week schedule of 6 hours/day, 5 days/week, with holidays and weekends off to simulate an industrial exposure regimen for man. Each exposure and control group consisted of 65 male and 65 female rats, 200 female mice, 100 male hamsters, and 8 dogs equally divided by sex. The numbers of rodents used were selected to provide a statistically valid number of each species after two years on study for comparison of exposure group and control incidence of histologic changes. Dogs were included in the study to provide indices of non-tumor effects, mainly from analyses of blood.

The exposure portion of this study continued for one year after which 20 mice, 10 rats and 10 hamsters from each group were necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents were held for an additional year and the dogs for 5 years of postexposure observation.

EXPERIMENTAL RESULTS

Exposure Measurements:

The exposure concentrations achieved during the 12-month exposure period are shown in Table 1. Exposure chamber concentration control was excellent after the first few weeks and is reflected in the relatively small standard deviations of the means.

TABLE 1. MEAN METHYLCYCLOHEXANE CONCENTRATIONS MEASURED IN ANIMAL EXPOSURE CHAMBERS

	Chamber 1	Chamber 2	Chamber 3	Chamber 4
No. of Sampling Days	243	243	243	243
Nominal Conc., ppm	400	400	2000	2000
Mean Measured Conc., ppm	401.5	398.9	2009	1998
Standard Deviations, ppm	± 4.5	± 2.5	± 46.6	± 52.4
Concentration Range, ppm	393-412	395-402	1878-2080	1847-2047

Growth:

Male rats exposed to both levels of MCH showed depressed growth throughout the study (Figure 1). Although the male rats showed an increase in weight gain after removal from the exposure chambers, they still did not attain the mean weight of the unexposed control group. The female rat weights were unaffected (Figure 2) during exposure as well as during the postexposure observation period. A definite depression in mean body weights was seen in the exposed hamster groups but was not dose related (Figure 3). Immediately following exposure, both exposed hamster groups gained weight and became equivalent to the control group.

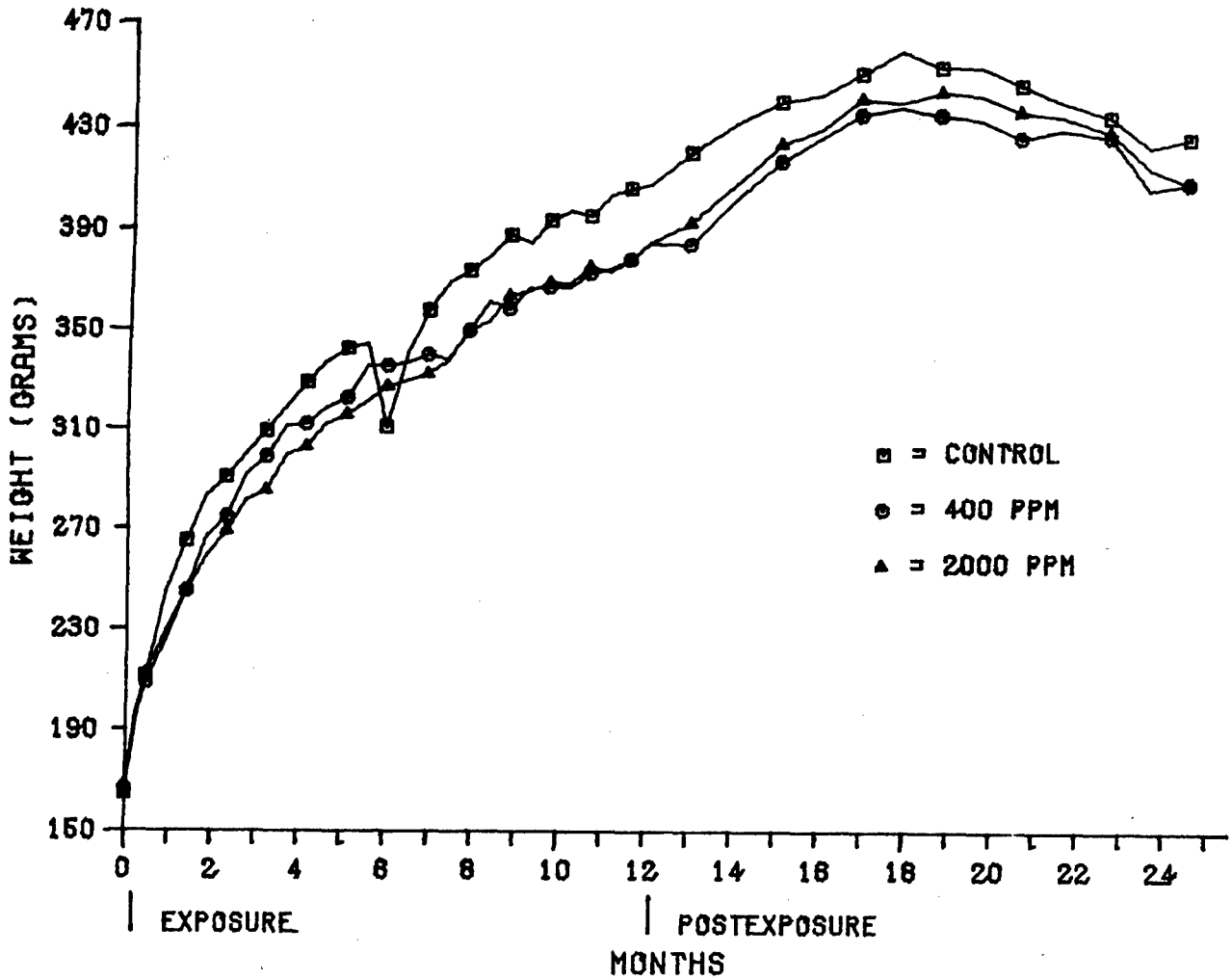

Figure 1. Mean body weights of male rats exposed to methylcyclohexane vapor for 12 months.

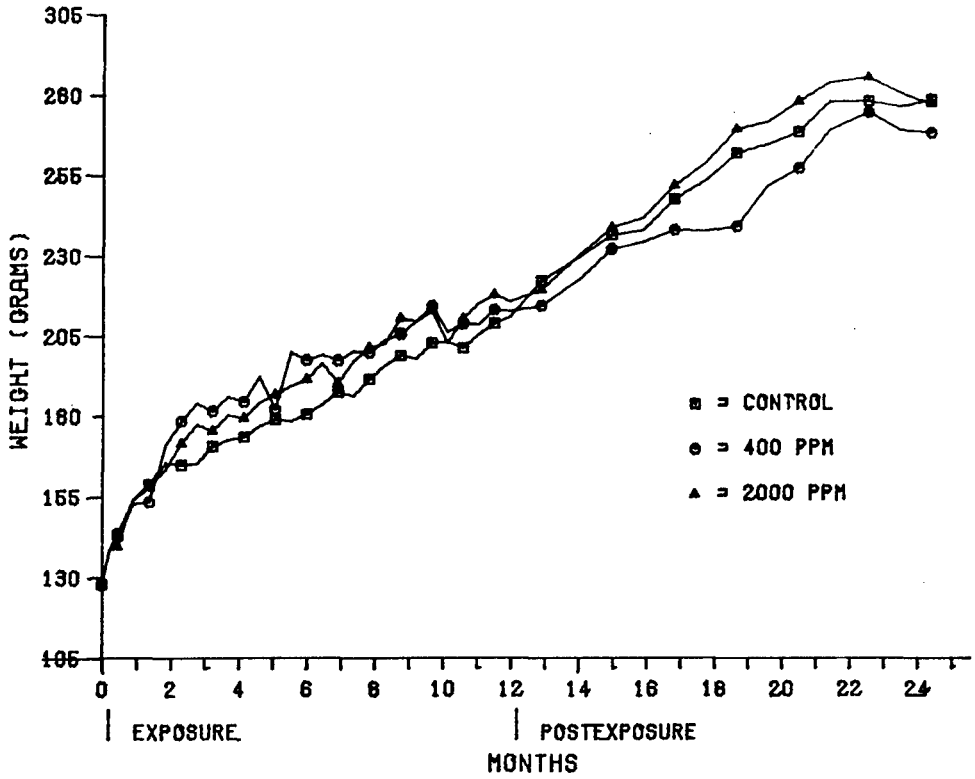

Figure 2. Mean body weights of female rats exposed to methylcyclohexane vapor for 12 months.

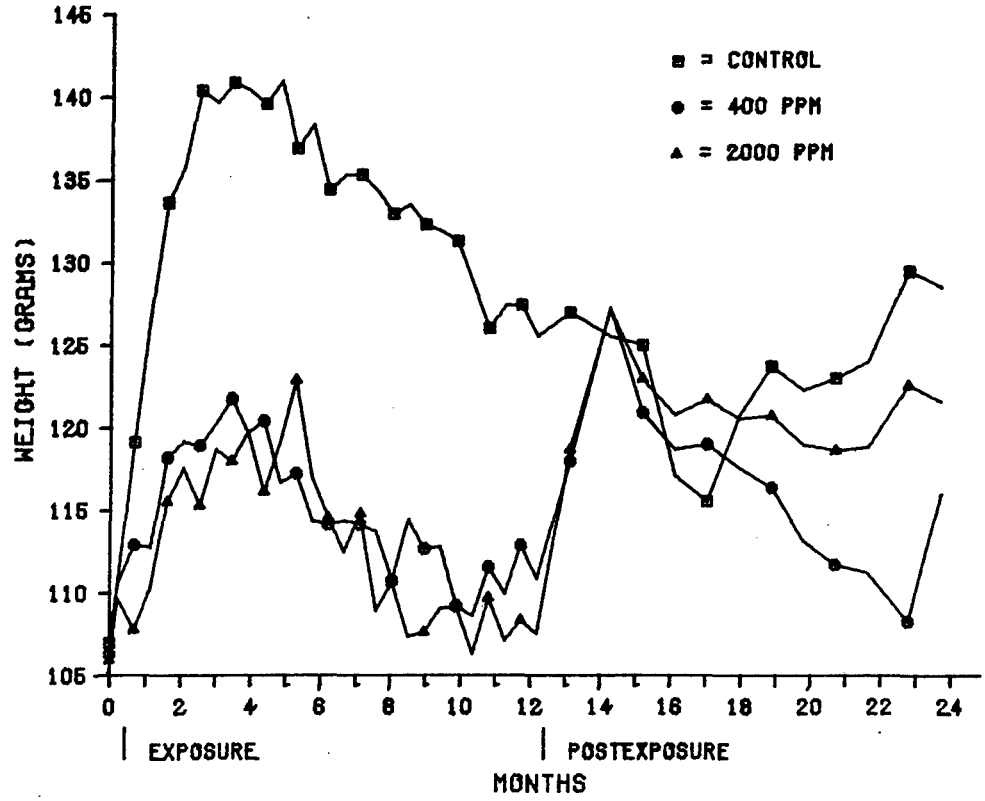

Figure 3. Mean body weights of male golden syrian hamsters exposed to methylcyclohexane vapor for 12 months.

Clinical Laboratory Measurements:

The hematology and clinical chemistry values of the male rats sacrificed after one year of exposure are shown in Table 2 and the hematology values for the female rats are listed in Table 3. There were no biologically significant differences between MCH exposed and control rats.

TABLE 2. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF MALE RATS[a] AFTER A ONE-YEAR INHALATION EXPOSURE TO MCH VAPOR

Parameter	Control	400 ppm	2000 ppm
RBC (10^6)	9.7	9.8	9.7
WBC (10^3)	6.7	5.4[b]	5.3[b]
HCT (%)	47.7	48.9[b,e]	47.0
HGB (gm/dl)	15.2	15.4	14.7[c,e]
Total Pro. (gm/dl)	7.2	7.3	7.3
Albumin (gm/dl)	4.2	4.2	4.1
Globulin (gm/dl)	3.0	3.1	3.1
Glucose (mg/dl)	162.8	170.3	165.8
Potassium (mEq/L)	5.3	6.0[b,d]	5.4
Calcium (mg/dl)	9.6	9.7	10.5
Sodium (mEq/L)	154.9	151.6[b]	150.1[c]
Bilirubin (mg/dl)	0.38	0.40	0.38
BUN (mg/dl)	14.2	15.4	14.4
Creatinine (mg/dl)	0.55	0.64[c,e]	0.58
SGPT (IU/l)	62.8	60.9	58.2
SGOT (IU/l)	91.6	94.7	86.4
Alk. Phos (IU/l)	12.5	11.8	9.7

[a] N = 9 or 10.
[b] Significantly different from controls, $p < 0.05$.
[c] Significantly different from controls, $p < 0.01$.
[d] Significantly different from other test group, $p < 0.05$.
[e] Significantly different from other test group, $p < 0.01$.

A significant increase in creatinine along with an increase in BUN was seen in the male group exposed to 400 ppm MCH but was not evident in the high exposure group. Low WBC's were found in all exposed groups, both male and female. Because of hemolysis in most samples of female rat blood, no clinical chemistry comparisons could be made.

TABLE 3. MEAN HEMATOLOGY VALUES OF FEMALE RATS AFTER A ONE-YEAR INHALATION EXPOSURE TO MCH VAPOR, N=10

Parameter	Control	400 ppm	2000 ppm
RBC (10^6)	7.8	7.8	7.9
WBC (10^3)	5.4	4.8	3.6[a]
HCT (%)	44.1	43.1	44.0
HGB (gm/dl)	14.5	14.4	14.3

[a] Significantly different from controls, $p < 0.01$.

Clinical determinations on dog blood taken at biweekly intervals gave variable but non-MCH related results. The only parameter affected by MCH exposure was a transient increase in the mean SGPT level of the dogs exposed to 2000 ppm which was caused by a single dog exhibiting a high SGPT level during the seventh exposure week while the other animals of the group were normal (Figure 4). The increase in SGPT values occurred again between 39 and 43 weeks in the same dog from the 2000 ppm group as well as in two dogs from the 400 ppm group. Significant differences in SGPT values of dogs were not seen during the five-year postexposure period.

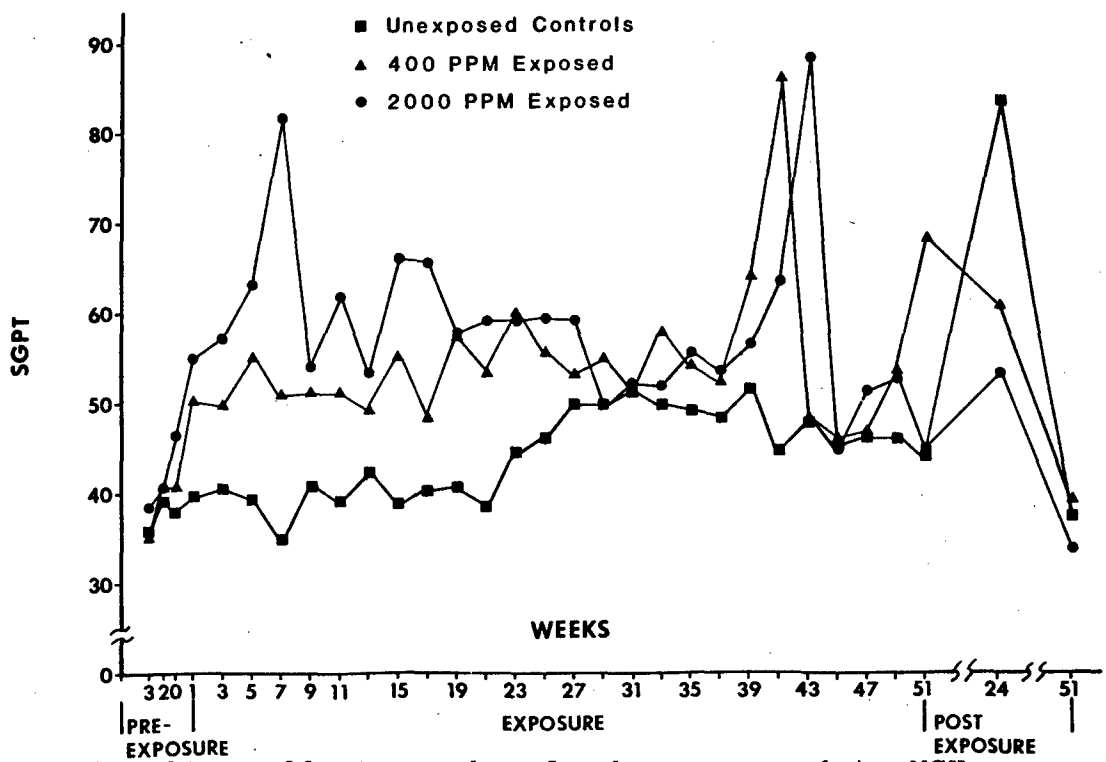

Figure 4. SGPT effects on beagle dogs exposed to MCH vapor for one year.

Pathology (Exposure Termination):

Micropathologic tissue changes seen in the rodents that died during and at the end of the 12-month intermittent exposure to MCH are listed in Tables 4-6.

Rats:

Since only a small number of lesions were observed in these animals, both tumors and nontumorous lesions are tabulated together. Only one tumor, a benign endometrial stromal polyp, was found in any female rat and this was seen in an animal exposed to 400 ppm MCH.

TABLE 4. TISSUE CHANGES SEEN IN MALE AND FEMALE FISCHER 344 RATS AT THE END OF 12-MONTH INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Controls	400 ppm	2000 ppm
Male Rats:			
Pituitary Adenoma	2	0	1
Testicular Tumor	0	5[a]	2
Adrenal Pheochromocytoma	1	1	0
Bile Duct Hyperplasia	1	2	0
Renal Tubular Dilatation	1	2	4
Lungs:			
Lymphocytic Infiltrates	2	0	1
Arterial Mineralization	2	1	0
Myocardial Fibrosis	2	3	0
Number of Animals Examined	11	10	11
Female Rats:			
Ovarian Cyst	0	4	2
Lungs:			
Lymphocytic Infiltrates	6	0	3
Arterial Mineralization	1	1	1
Endometrial Stromal Polyp	0	1	0
Number of Animals Examined	11	10	10

[a] Statistically different from control incidence at $p < 0.05$.

The tumors seen in the male rats are commonly found in this strain. There appeared to be a slight increase in dilatation of renal tubules in the 2000 ppm MCH exposed male rats but no other indication of kidney injury was seen at the end of the 12-month exposure.

Mice:

Lesions seen in the mice following the 12-month exposure occurred at much the same incidence in all groups.

Hamsters:

Only one tumor, a benign tumor of an adrenal gland in an animal from the high exposure group was seen in all hamsters examined. The other lesions seen were fairly uniform in all groups and not related to MCH exposure.

TABLE 5. TISSUE CHANGES SEEN IN FEMALE C57BL/6 MICE AT THE END OF 12-MONTH INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Controls	400 ppm	2000 ppm
Lung:			
Lymphoid Hyperplasia	1	4	3
Alveolar Bronchiolar Adenoma	1	0	0
Liver:			
Fatty change	10	7	5
Hyperplasia	3	4	1
Malignant Lymphoma	3	4	4
Pituitary Adenoma	3	0	0
Uterine Cysts	18	16	14
Kidney Hyperplasia	5	4	2
Number of Animals Examined	29	35	39

TABLE 6. TISSUE CHANGES SEEN IN MALE GOLDEN SYRIAN HAMSTERS AT THE END OF 12-MONTH INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Controls	400 ppm	2000 ppm
Kidney Mineralization	5	4	1
Renal Tubular Dilatation	7	1	3
Pituitary Fatty Change	2	4	3
Adrenal Adenoma	0	0	1
Number of Animals Examined	24	20	17

Pathology (Termination):

The results of examination of tissue from the animals that died during the postexposure observation period or were killed at the study termination are listed in Tables 7 through 14. The tables of non-neoplastic lesions of the four species have been abbreviated to exclude lesions of very low incidence.

TABLE 7. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Controls	400 ppm	2000 ppm
Males			
Liver			
Bile Duct Hyperplasia	32/53	22/55	19/52
Necrosis	2/53	0/55	1/52
Circulatory System			
Myocardial Fibrosis	11/53	3/55	14/52
Pulmonary Artery Mineralization	6/53	3/55	0/52
Kidney			
Medullary Mineralization	1/53	2/55	19/52[b]
Nephropathy	49/53	52/55	52/52
Papillary Hyperplasia	1/53	1/55	23/52[b]
Tubular Degeneration	1/53	0/55	2/52
Testes			
Atrophy	4/53	2/55	1/52
Lungs			
Adenomatosis	1/53	2/55	0/52
Females			
Liver			
Bile Duct Hyperplasia	5/52	2/50	3/54
Necrosis	4/52	0/50	1/54
Circulatory System			
Myocardial Fibrosis	1/52	3/51	4/53
Pulmonary Artery Mineralization	6/52	2/51	3/54
Kidney			
Medullary Mineralization	4/52	0/51	1/54
Nephropathy	15/52	7/51	15/54
Reproductive			
Ovarian Cysts	6/50	2/51	3/52
Uterine Dilatation	5/52	9/51	4/52
Mammary Gland			
Cystic Hyperplasia	10/47	17/53	14/48
Lungs			
Adenomatosis	2/52	0/51	1/54

[a] Number of lesions observed/number of animals examined.
[b] Statistically different from control incidence at $p < 0.01$.

TABLE 8. NEOPLASTIC LESIONS[a] SEEN IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Keratoacanthoma	0/51	1/55	3/52
Fibroma	3/53	4/55	0/52
Fibroadenoma	0/53	1/55	0/52
Osteosarcoma	1/53	0/55	0/52
Basal Cell Tumor	0/53	1/55	1/52
Mammary Gland Fibroadenoma	0/46	0/47	2/52
Myxoma	1/53	0/55	0/52
Lungs			
Squamous Cell Carcinoma	0/54	1/55	0/52
Nasal			
Squamous Cell Carcinoma	1/53	0/55	0/52
Liver			
Mononuclear Cell Leukemia	0/53	0/55	0/52
Pituitary			
Adenoma	17/51	11/54	16/48
Carcinoma	2/51	1/54	0/48
Neoplasm	1/51	0/54	0/48
Thyroid			
Adenoma	4/52	5/54	5/51
Carcinoma	0/52	1/54	2/51
Kidney			
Renal Cell Adenoma	0/54	0/55	1/52
Renal Cell Carcinoma	0/54	1/55	0/52
Adrenals			
Adenoma	1/54	1/55	5/52
Carcinoma	0/54	1/55	0/52
Pheochromocytoma	3/54	0/55	2/52
Stomach			
Leiomyoma	0/53	0/54	1/52
Pancreas			
Islet Cell Adenoma	1/53	1/54	1/51
Testis			
Interstitial Cell Tumor	49/54	49/55	50/52
Zymbals Gland			
Squamous Cell Carcinoma	0/54	0/55	1/52
Preputial Gland			
Adenocarcinoma	0/54	0/55	1/52
Parathyroid			
Adenoma	1/54	0/55	0/52
Multiple Organ			
Mesothelioma	1/54	1/55	1/52
Malignant Lymphoma	1/54	2/55	0/52
Bronchial Mucous Gland			
Adenoma	0/54	1/55	0/52
Histiocytic Leukemia	0/54	3/55	2/52

[a] Number of lesions observed/number of animals examined.

TABLE 9. NEOPLASTIC LESIONS[a] SEEN IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin			
Keratoacanthoma	0/49	0/54	2/51
Fibroma	1/52	0/51	3/51
Trichoepithelioma	1/52	0/51	0/51
Fibroadenoma	1/52	3/51	3/51
Adenoma	1/52	0/51	0/51
Sarcoma, Undifferentiated	0/52	1/51	0/51
Sarcoma	0/52	1/51	0/51
Mammary Gland Fibroadenoma	0/47	4/50	6/48
Lungs			
Alveolar/Bronchiolar Carcinoma	0/52	1/54	0/54
Osteosarcoma	0/52	0/54	1/54
Sarcoma	0/52	1/54	0/54
Pituitary			
Adenoma	11/50	16/50	17/54
Carcinoma	3/50	4/50	5/54
Thyroid			
Adenoma	1/52	1/52	2/51
Carcinoma	2/52	3/52	1/51
Parathyroid			
Adenoma	0/31	1/40	0/35
Mediastinal Lymph Node			
C-Cell Carcinoma	0/52	1/54	0/54
Adrenals			
Adenoma	0/52	1/53	1/54
Adenocarcinoma	1/52	0/53	0/54
Pancreas			
Adenocarcinoma	1/51	0/54	0/50
Uterus			
Endometrial Stromal Polyp	7/52	4/54	0/52
Adenocarcinoma	3/52	0/54	0/52
Leiomyosarcoma	0/52	1/54	0/52
Urinary Bladder			
Adenocarcinoma	1/45	0/53	0/51
Brain			
Astrocytoma	0/52	1/54	0/53
Clitoral Gland			
Adenoma	2/52	0/54	0/53
Abdominal Cavity			
Lipoma	1/52	0/54	1/53
Adenocarcinoma	1/52	0/54	3/53
Mesothelioma	0/52	1/54	0/53
Myxosarcoma	0/52	0/54	1/53
Circulatory System			
Histiocytic Leukemia	2/52	2/54	5/53
Malignant Lymphoma	1/52	0/54	0/53

[a] Number of lesions observed/number of animals examined.

TABLE 10. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Lungs			
Alveolar Crystals	24/170	8/158	6/155
Alveolar Macrophages	31/170	17/158	9/155
Perivascular Cuffing	20/170	25/158	19/155
Lymphoid Hyperplasia	20/170	23/158	25/155
Spleen			
Hematopoiesis	22/164	34/150	23/154
Liver			
Fatty Change	36/171	14/159	16/155
Hematopoiesis	20/171	20/159	24/155
Duodenum			
Mesentery Strangulation	19/167	7/150	10/151
Kidney			
Hydronephrosis	10/171	13/159	7/155
Perivascular Cuffing	13/171	14/159	8/155
Uterus			
Multiple Cysts	10/164	22/158	23/152
Endometrial Dilatation	30/164	38/158	29/152
Ovaries			
Cysts	19/149	20/155	24/135
Hemorrhagic Cysts	12/149	14/155	12/135
Mammary Gland			
Cystic Hyperplasia	26/145	10/118	12/119
Thyroid Gland			
Papillary Hyperplasia	79/164	39/151	44/145

[a] Number of lesions observed/number of animals examined.

TABLE 11. NEOPLASTIC LESIONS[a] SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Keratoacanthoma	0/167	0/154	1/150
Fibroma	1/167	0/154	0/150
Lung			
Alveolar/Bronchiolar Adenoma	4/170	6/158	0/155
Alveolar/Bronchiolar Carcinoma	3/170	1/158	3/155
Lymph Node			
Hemangiosarcoma	1/166	0/150	0/146
Heart			
Carcinoma	1/170	0/158	0/155
Liver			
Hepatocellular Adenoma	0/171	1/152	0/152
Hemangiosarcoma	0/171	0/152	1/152
Duodenum			
Papilloma	1/167	0/150	0/151
Papillary Adenoma	1/167	1/150	0/151
Uterus			
Neoplasm	1/164	0/158	1/152
Leiomyosarcoma	0/164	1/158	1/152
Ovaries			
Adenoma	1/149	0/155	0/135
Tubular Adenoma	1/149	1/155	4/135
Pituitary			
Adenoma	72/142	40/142	44/118
Carcinoma	4/142	0/142	0/118
Adenocarcinoma	5/142	0/142	2/118
Adrenal			
Adenoma	0/170	0/158	1/149
Thyroid			
Adenoma	0/164	1/151	0/145
Follicular-Cell Adenoma	2/164	1/151	1/145
Lacrimal Gland			
Adenoma	0/171	1/162	0/155
Bone			
Osteosarcoma	0/162	1/159	0/151
Circulatory System			
Malignant Lymphoma	45/171	44/162	56/155
Leukemia	0/171	0/162	1/155

[a] Number of lesions observed/number of animals examined.

TABLE 12. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN MALE HAMSTERS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Kidney			
Cortical Fibrosis	4/75	12/76	10/81
Mineralization, Collecting Tubules	26/75	19/76	24/81
Mineralization, Convoluted Tubules	14/75	10/76	8/81
Dilatation, Convoluted Tubules	17/75	16/76	17/81
Mineralization, Renal Pelvis	10/75	0/76	6/81
Testis			
Atrophy	4/76	3/76	2/81
Aspermatogenesis	3/76	4/76	5/81
Adrenal Gland			
Cortical Hyperplasia	35/75	30/76	29/80

[a] Number of lesions observed/number of animals examined.

Rats:

In male rats, the major target organ was the kidney where two types of lesions were associated with exposure. Virtually all of the male rats had lesions consistent with progressive renal nephropathy, common in older male rats. In the male rats exposed to the higher level, there was a statistically significant increase in the occurrence of medullary mineralization and epithelial hyperplasia of the renal papilla. However, no increase of these lesions over controls was seen in the group exposed to 400 ppm. Interstitial cell tumors of the testes, seen at study termination, appeared to be equally distributed between the test and control groups and not related to exposure. No dose related lesions were noted in the exposed female rats when compared to the control group.

Mice, Hamsters, and Dogs:

No significant lesions were found in female mice, male hamsters, or beagle dogs when compared to their respective control groups. Lesions noted were those commonly seen in older animals of these groups.

Neoplastic changes seen in rats, mice, and hamsters were those expected in aging animals of these species. No neoplastic lesions were found in dogs. Statistical analysis of the data failed to indicate any significant increase in tumor formation in the MCH exposed animals when compared to their respective controls.

TABLE 13. NEOPLASTIC LESIONS[a] SEEN IN MALE HAMSTERS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Fibroma	0/74	0/76	1/80
Trachea			
Adenoma	0/72	0/75	2/79
Spleen			
Hemangiosarcoma	0/71	1/75	1/78
Lymph Node			
Neoplasm	1/73	0/74	0/78
Liver			
Carcinoma	0/74	0/77	1/80
Islet-Cell Carcinoma, metastatic	0/74	1/77	0/80
Hepatocellular Carcinoma	0/74	1/77	0/80
Hemangiosarcoma	0/74	1/77	1/80
Angioma	0/74	1/77	0/80
Pancreas			
Islet-Cell Carcinoma	0/63	1/72	1/75
Duodenum			
Undifferentiated Sarcoma	0/71	0/76	1/79
Kidneys			
Renal-Cell Carcinoma	0/75	1/76	0/81
Adrenal Gland			
Carcinoma	2/75	3/76	7/80
Adenoma	18/75	21/76	12/80
Adenocarcinoma	1/75	0/76	0/80
Pheochromocytoma	0/75	1/76	0/80
Thyroid			
C-Cell Adenoma	0/71	0/67	1/71
C-Cell Carcinoma	2/71	1/67	0/71
Parathyroid			
Adenoma	1/45	0/43	1/47
Multiple Organs			
Carcinoma	0/76	0/77	1/82
Sarcoma	0/76	0/77	1/82
Malignant Lymphoma	2/76	5/77	4/82
Myelogenous Leukemia	0/76	1/77	0/82

[a] Number of lesions observed/number of animals examined.

TABLE 14. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN BEAGLE DOGS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Thyroid			
Follicular Hyperplasia	2/8	0/8	3/8
Inflammation, Chronic	2/8	1/8	1/8
Gallbladder			
Microcystic Degeneration	2/8	3/8	3/8
Spleen			
Siderotic Nodule	2/8	1/8	4/8

[a] Number of lesions observed/Number of animals examined.

Discussion:

The only significant toxic effect of chronic exposure to inhaled methylcyclohexane found was renal change in male rats. The exposure related renal injury was not seen in female rats, female mice, male hamsters, or in either sex of beagle dog. Only male rats exposed to 2000 ppm MCH had significantly greater incidence of renal tubular dilation at exposure termination, and progression of renal pathology to papillary hyperplasia and medullary mineralization occurred only in the group exposed to the higher level. These findings are consistent with those produced by other paraffinic, cycloparaffinic, and alkylaromatic hydrocarbons. This syndrome now referred to as hydrocarbon nephropathy, is characterized by an increase in the incidence of hyalin droplets and of regenerative tubular epithelia in the cortex. In its most severe form the tubules at the corticomedullary junction become dilated and filled with proteinaceous debris with some necrosis.

The incidence and severity of the hydrocarbon nephropathy seen after chronic MCH inhalation exposure was much less than that reported for gasoline (MacFarland, 1983), decalin (Gaworski et al., 1979), and for petroleum and shale derived JP-5 fuels (Gaworski et al., 1982) even though exposure levels were greater with MCH. Moreover, these previous studies had not shown any level of hydrocarbon exposure that did not cause kidney pathology. In this study, 400 ppm MCH had no pathologic effects on male rat kidney that could be distinguished from controls.

Under the conditions of this inhalation study, exposure to 2000 ppm methylcyclohexane (8000 mg/m^3) produced no tumors. Similar exposure to inhaled JP-10 (MacEwen and Vernot, 1983) was shown to produce severe hydrocarbon nephropathy and renal carcinoma in 18% of the male Fischer 344 rats held one year postexposure after 12 months inhalation exposure to 100 ppm JP-10 (tricyclodecane). Inhalation exposure to 2000 ppm gasoline also produced renal carcinomas (Kitchen, 1983) in Fischer 344 rats.

The results of this study indicate no increase in tumor formation in exposed animals when compared to their untreated controls. The tumors seen in all groups were those common to the species.

The results of this study support the selection and safety of the current TLV of 400 ppm for methylcyclohexane.

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